Biomarkers, 2009; 14(7): 465–472

informa
healthcare

SHORT COMMUNICATION

Assessment of oxidative stress in coronary artery bypass surgery: comparison between the global index OXY-SCORE and individual biomarkers

Fabrizio Veglia[1], José P. Werba[1], Elena Tremoli[1,2], Isabella Squellerio[1], Erminio Sisillo[1], Alessandro Parolari[1,3], Fabiana Minardi[1], and Viviana Cavalca[1,3]

[1]*Centro Cardiologico Monzino IRCCS, Milan, Italy,* [2]*Department of Pharmacological Sciences, University of Milan, Milan, Italy, and* [3]*Institute of Cardiology, University of Milan, Milan, Italy*

Abstract

The performances of the OXY-SCORE, a summary index of oxidative stress, and of its individual components (plasma malondialdehyde (MDA), oxidized and reduced glutathione, individual antioxidant capacity, α- and γ-tocopherol and urinary isoprostanes) were assessed in 47 patients undergoing coronary surgery, randomly assigned to cardiopulmonary bypass (CPB) or off-pump procedure (OPCAB) associated with less oxidative stress. The ability of the OXY-SCORE to classify correctly the patients was high (area under the ROC curve 0.90). Only free MDA showed a similar performance, but it was insensitive to the minor variations of the oxidative balance in the OPCAB group.

Keywords: *Malondialdehyde; glutathione; oxidative stress global index; coronary artery bypass graft*

Introduction

Oxidative stress is usually defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defences, which leads to oxidative damage and dysfunction of macromolecules, cells and tissues (Droge 2002, Valko et al. 2007). Studies aimed at assessing oxidative stress or changes in oxidative status in different environmental, clinical or pharmacological conditions, have generally been performed by estimating the consumption of a specific antioxidant (i.e. tocopherol, glutathione, etc.), or the increase of antioxidant enzymes, mass or activity (superoxide dismutase, catalase, glutathione peroxidase, etc.), or the products of oxidative damage to biological molecules (malondialdehyde (MDA), isoprostanes, nitrotyrosine, 8-hydroxy 2'-deoxyguanosine, etc.) (Cherubini et al. 2005, Dalle-Donne et al. 2006, Veglia et al. 2006).

Our group has, recently, proposed the OXY-SCORE, a comprehensive index of oxidative stress derived from a computation of different variables relevant for the oxidative balance (Veglia et al. 2006). The underlying principle of the OXY-SCORE is analogous to that of some cardiovascular risk equations, which integrate risk factors (i.e. cholesterol levels, blood pressure, etc.), protective factors (i.e. high-density lipoprotein-cholesterol) and markers of organ damage (i.e. microalbuminuria, left ventricular hypertrophy, etc.) to obtain a single number (i.e. Framingham risk score, Procam score, etc.) which reflects a comprehensive index of risk. We have previously reported that the OXY-SCORE is more sensitive than individual oxidative markers in detecting even slight differences in oxidative status between age groups and gender in healthy subjects (Veglia et al. 2006).

The objective of the present study was to compare the performance of OXY-SCORE with those of its individual components in a clinical scenario characterized by significant acute changes in oxidative stress. With this aim, the OXY-SCORE was computed using a panel of oxidative markers that had been evaluated in a previous study.

Address for Correspondence: Fabrizio Veglia, Unit of Biostatistics, Centro Cardiologico Monzino IRCCS, Via Parea, 4, 20138 Milano, Italy. Tel.: +39 02 5800 2260. Fax: +39 02 5800 2623. E-mail: fabrizio.veglia@ccfm.it

(Received 21 May 2009; revised 22 June 2009; accepted 22 June 2009)

ISSN 1354-750X print/ISSN 1366-5804 online © 2009 Informa UK Ltd
DOI: 10.3109/13547500903134395

We reanalysed data from patients subjected to coronary artery bypass graft surgery with or without utilization of cardiopulmonary bypass (CPB or OPCAB, respectively), two clinical circumstances with patent differences in the fluctuations of the oxidative balance. Specifically, we have previously reported a marked increase of free MDA and isoprostanes in CPB but not in OPCAB patients (Cavalca et al. 2006). A reduced postoperative systemic inflammatory and oxidative response in OPCAB, compared with CPB, was also reported by others (Gerritsen et al. 2001, Matata et al. 2000, Biglioli et al. 2003), a phenomenon possibly explained by blood cells stress and platelet activation during contact with the material surface of the heart–lung machine in CPB.

Methods

Patients

The characteristics of the patients included in this study have been described in detail elsewhere (Cavalca et al. 2006). Briefly, 50 first-time isolated low-risk (EuroSCORE <6) coronary bypass surgery patients were enrolled in accordance with a protocol approved by the Institutional Review Board at the Centro Cardiologico Monzino. Written informed consent was obtained from each patient. Exclusion criteria were intake of vitamin supplements or drugs with antioxidant properties, Q-wave myocardial infarction in the last 6 weeks, unstable angina or poor left ventricular function (ejection fraction (EF) <0.30).

Patients for whom both an on-pump or an off-pump procedure were deemed technically feasible, were randomly assigned to CPB ($n=25$) or OPCAB ($n=25$). The same surgical and anaesthetic team managed all patients. The surgical procedure was described in detail elsewhere (Cavalca et al. 2006). Briefly, after midline sternotomy and internal mammary harvesting, heparin (300 IU kg^{-1}) was given and activated clotting time was maintained at 440 s or greater with additional heparin in both groups. A non-pulsatile roller pump and hollow-fibre oxygenators were used in CPB. Cardiopulmonary bypass was initiated with cannulas placed in the ascending aorta and right atrium (two-stage venous cannula). Each operation was performed with tepid hypothermia (32–33°C) and haemodilution. During CPB, blood flow was maintained at 2.4 l min^{-1} m^{-2}. Myocardial protection was achieved by the administration of cool, antegrade and retrograde multidose blood cardioplegia. In OPCAB, mechanical stability of the coronary arteriotomy area was achieved with the Octopus IV system (Medtronic Inc., Minneapolis, MN, USA) and a soft plastic coronary flow-shunt was passed into the coronary arteriotomy to reduce myocardial ischaemia and to improve visualization of the anastomosis field. The haemodynamic management of patients during distal coronary anastomosis consisted mainly in the careful and progressive elevation of the heart with tissue slices, associated with substantial volume administration to allow the heart to adapt to the new positioning and to avoid major haemodynamic derangements or need of inotropic drug administration. Upon completion of distal and proximal coronary anastomoses, heparin was antagonized with protamine sulfate at a 1:1 ratio.

Samples collection

Blood samples were collected from radial artery into tubes containing ethylenediaminetetraacetic acid (EDTA, 9.3 mM; Vacutainer Systems, Becton Dickinson, Rutherford, NY, USA) as anticoagulant at seven time points: before induction of anaesthesia (T0, baseline), after sternotomy (T1), 45 min after heparin administration (T2), after protamine administration (T3), at the end of surgery (T4, about 1.5 h from T2), 4 h after the arrival to the intensive care unit (T5), and 24 h after surgery (T6).

Five hundred microlitres of blood were immediately precipitated with 500 μl of 10% trichloroacetic acid in 1 mmol l^{-1} EDTA solution and stored at –80°C (whole blood samples). The remaining blood sample was centrifuged (3000*g* for 10 min at 4°C) within 30 min from drawing to obtain plasma, which was stored at –80°C until analysis. Urine was collected the night before surgery (pre), 4–6 h after the beginning of surgery (during) and overnight 24 h after surgery (post). All the urine samples, added with the antioxidant 4-hydroxy-tempo (1 mmol l^{-1}; Sigma-Aldrich Chemical Co., St Louis, MO, USA), were stored at –80°C until analysis.

Biochemical analytes detection

Plasma free and total MDA (F-MDA and T-MDA) levels were determined by the reference method based on a gas chromatography–mass spectrometry (GC-MS) technique, with synthesized dideuterated MDA added as the internal standard (Cighetti et al. 1999). T-MDA was evaluated after alkaline hydrolysis (NaOH 1 mol l^{-1}) at 60°C before the fluorogenic derivatization with *ortho*-phthaldehyde. The intra- and interassay coefficients of variation (CVs) were 1.2 and 1.5% for F-MDA and 2.0 and 2.1% for T-MDA, respectively.

Isoprostane $iPF_2\alpha$-III levels were determined in urine by means of a previously described enzyme immunoassay method (Wang et al. 1995) using a commercially available kit (SPI-BIO, Saclay F; Cayman Chemical Co., Ann Arbor, MI, USA), after a two-step organic extraction onto SPE cartridges. To correct for $iPF_2\alpha$-III losses during organic extraction, 20 000 dpm of [3H8]-$PGF_2\alpha$ were added to the urine samples (2 ml)

before any manipulations. Acetic acid (60 µl) was added to acidify the samples to pH 6.0. The samples were centrifuged (2500*g* for 15 min) and the supernatants were passed through the Supelclean LC-18 cartridge (Supelco, Bellafonte, PA, USA), conditioned with 1 ml of methanol and 1 ml of water, washed with 1 ml of water, 1 ml of hexane, 1 ml of a 75/25 v/v hexane/ethyl acetate solution and eluted with 1 ml of ethyl acetate. The eluates were diluted with 1 ml of hexane and loaded onto a Supelchem LC-Si cartridge (Supelco) conditioned with 1 ml of ethyl acetate and 1 ml of a 50/50 v/v hexane/ethyl acetate solution and eluted with 1 ml of a 20/80 v/v methanol/ethyl acetate solution. Final eluates were evaporated under vacuum (Savant, Farmingdale, NY, USA) and reconstituted with 1.8 ml of enzyme-immunoassay buffer (phosphate buffer 0.1 M, pH 7.4 containing 23.4 g l^{-1} NaCl, 1 g l^{-1} BSA, 0.1 g l^{-1} NaN_3, 0.372 g l^{-1} EDTA). The $iPF_2\alpha$-III value in each sample was corrected for the extent of [3H8]-$PGF_2\alpha$ losses during organic extraction and expressed as pmol $mmol^{-1}$ creatinine, measured by standard methods using the Jaffe's reaction. The intra- and interassay CVs were 4.9 and 10.2%, respectively.

Whole blood GSH and GSSG levels were measured by high-performance liquid chromatography (HPLC) technique on a C18 RP column after centrifugation at 14 000*g* for 10 min at 4°C, as previously reported (Veglia et al. 2006). The intra- and interassay CVs were 1.1 and 5.9% for GSH, and 3.9 and 12.5% for GSSG, respectively.

Vitamin E, i.e. α-tocopherol (α-TH) and γ-tocopherol (γ-TH) concentrations, were detected in plasma by HPLC after organic extraction as previously described (Werba et al. 2007). The intra- and interassay CVs were 3.3 and 4.0% for plasma α-TH, and 3.3 and 4.7% for γ-TH, respectively.

Plasma individual antioxidant capacity (IAC) was assessed using a commercially available spectrophotometric assay (OXY-adsorbent test, Diacron, GR, Italy). The intra- and interassay CVs were 2.2 and 6.3%, respectively.

Oxidative score

The details for the computation of the OXY-SCORE are reported elsewhere (Veglia et al. 2006). Briefly, it was calculated as the average of the standardized pro-oxidant factors (F-MDA, T-MDA, $iPF_2\alpha$-III and GSSG) minus the average of the standardized antioxidant variables (α-TH, γ-TH, GSH and IAC). Variables with skewed distribution (F-MDA, T-MDA, $iPF_2\alpha$-III and GSSG) were log-transformed before computation. The value of the OXY-SCORE is approximately zero when the levels of all the analytes are near the average of normal values or high levels of damage biomarkers are compensated by high levels of antioxidant defences.

Statistical analysis

The present study was a reanalysis of data from 47 patients. This sample size allowed approximately a 90% power to detect as significant, with a 0.05 alpha level, a between-group difference of at least one standard deviation, at any time point. In the case of the OXY-SCORE, this corresponds to 1.46 units. Numerical variables are presented as means and 95% confidence intervals, categorical variables as absolute and percentage frequency. The time courses of the analytes were compared by repeated measures covariance analysis with a group × time factorial design. Within groups variations versus baseline

Table 1. Demographic and clinical characteristics of the patients.

	CPB	OPCAB	
	n = 25	*n* = 22	*p*-Value
Age (years), mean ± SD	63.4 ± 11.5	68.4 ± 8.9	0.11
Men, *n* (%)	17 (68)	18 (82)	0.33
BMI, mean ± SD	24.6 ± 3.3	25.6 ± 2.8	0.35
Hypertension, *n* (%)	16 (64)	12 (55)	0.72
Diabetes, *n* (%)	7 (28)	5 (23)	0.94
Hypercholesterolaemia, *n* (%)	18 (86)	18 (78)	0.37
Ejection fraction (%)	56.2 ± 13.4	55.8 ± 9.6	0.91
EuroSCORE, median (inter-quartile range)	4 (3–6)	4 (0–5)	0.75[a]
NYHA I/II, *n* (%)	23 (92)	21 (95)	1.0[b]
NYHA III/IV, *n* (%)	2 (8)	1 (5)	
Previous MI, *n* (%)	12 (48)	8 (36)	0.61
COPD, *n* (%)	1 (5)	2 (8)	>0.99
CRF, *n* (%)	3 (14)	0(0)	0.24

The two groups were compared by two-sample *t*-test.
[a]Data were log-transformed before analysis. [b]Data were compared by Fisher's exact test.
CPB, cardiopulmonary bypass; OPCAB, off-pump procedure; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; MI, myocardial infarction.

Table 2. Patients' biochemical values in plasma at baseline.

	CPB	OPCAB	
	n = 25	*n* = 22	*p*-Value
$iPF_2\alpha$-III (pmol $mmol^{-1}$ creatinine), median (IQR)	99.5 (56–128)	98.5 (67–155)	0.50[a]
F-MDA (µmol l^{-1})	0.83 ± 0.42	1.04 ± 0.55	0.14
T-MDA (µmol l^{-1})	5.55 ± 1.38	5.19 ± 1.04	0.33
GSSG (µmol gHb^{-1})	0.3 ± 0.19	0.36 ± 0.22	0.52
GSH (µmol gHb^{-1})	4.47 ± 1.35	4.45 ± 1.36	0.97
α-TH (µg ml^{-1})	15.27 ± 3.51	15.64 ± 4.87	0.77
γ-TH (µg ml^{-1})	0.76 ± 0.26	0.79 ± 0.33	0.68
IAC (µmol HClO ml^{-1})	285 ± 49.9	280.5 ± 36.8	0.73
OXY-SCORE	0.27 ± 0.65	0.49 ± 0.73	0.29

Values are mean ± SD, unless otherwise indicated. The two groups were compared by two-sample *t*-test, or [a]Wilcoxon rank-sum test.
CPB, cardiopulmonary bypass; OPCAB, off-pump procedure; IQR, interquartile range; MDA, malondialdehyde; IAC, individual antioxidant capacity; TH, tocopherols.

were assessed by paired *t*-test, and *p*-values were corrected for multiple testing by the Bonferroni method. The ability of individual markers and of OXY-SCORE to discriminate between the two clinical situations was assessed by ROC curve analysis. *p*-Values below 0.05 were considered as statistically significant. All tests were two-sided and were performed using the SAS statistical package (SAS Institute Inc., Cary, NC, USA).

Results

Fifty patients undergoing cardiac surgery were assessed for eligibility and randomly assigned to the CPB or OPCAB groups. Three OPCAB patients were excluded from the study for refused surgery after randomization (one patient), conversion to CPB (one patient) and perioperative myocardial infarction (one patient). Patients' characteristics are reported in Table 1.

Patients assigned to CPB or OPCAB were comparable for age, gender, body mass index (BMI), risk factors and EuroSCORE (Table 1).

Time course

Levels of individual oxidative markers and the OXY-SCORE did not significantly differ between the two groups at baseline (Table 2). The time course and the

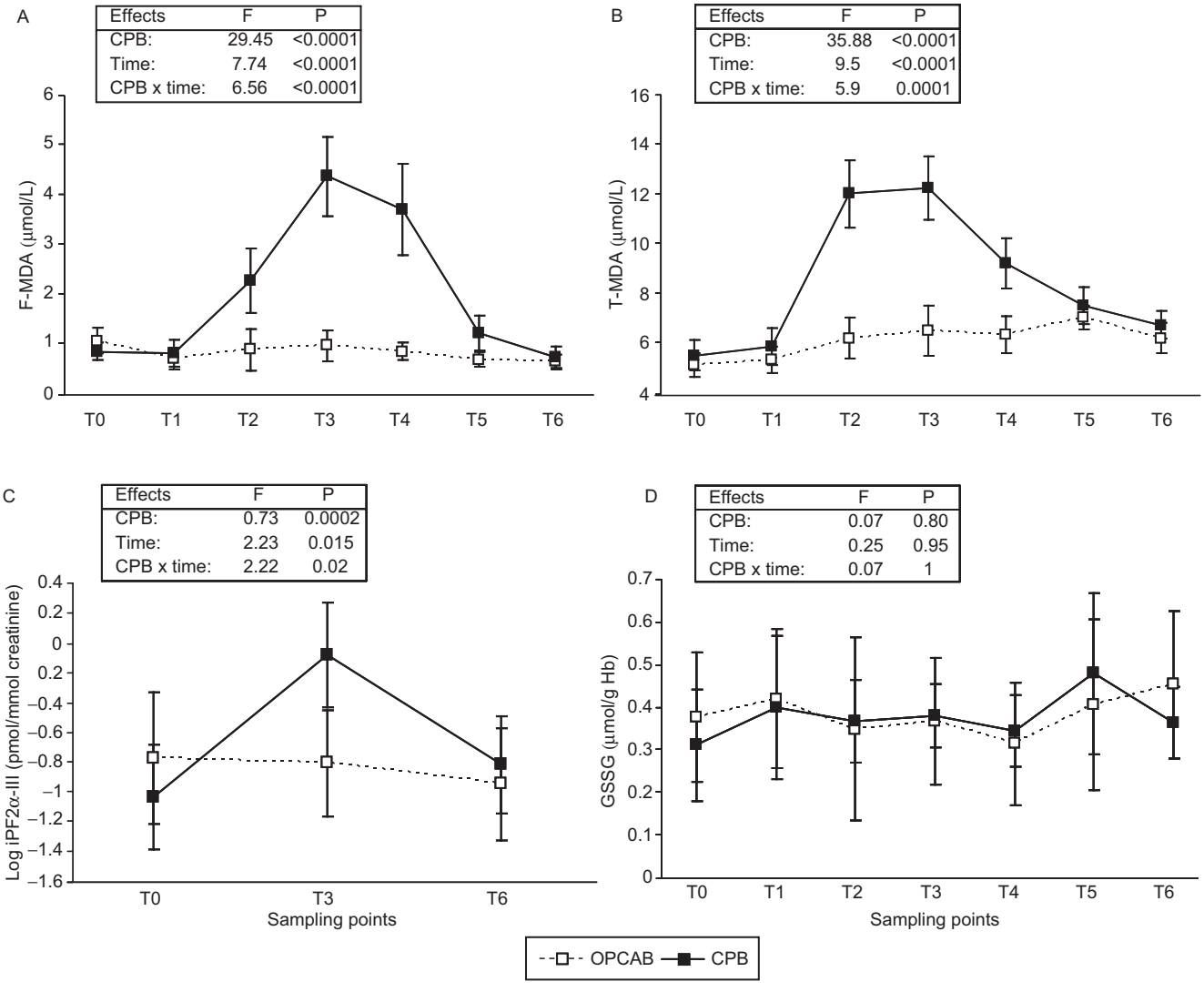

Figure 1. Time course of pro-oxidant biomarkers: free malondialdehyde (F-MDA, panel A), total malondialdehyde (T-MDA, panel B), isoprostanes ($iPF_2\alpha$-III, panel C) and oxidized glutathione (GSSG, panel D). Filled squares, solid line, cardiopulmonary bypass (CPB); empty squares, dotted line, off-pump procedure (OPCAB). Sampling points: T0, baseline; T1, after sternotomy; T2, 45 min after heparin administration; T3, after protamine administration; T4, at the end of surgery; T5, 4 h after arrival in the intensive care unit; and T6, 24 h after surgery. The values are expressed as means and 95% confidence intervals. Values of $iPF_2\alpha$-III are log-transformed. Inserts show *F* and *p*-values for the effects of procedure (CPB), time and interaction (CPB × time).

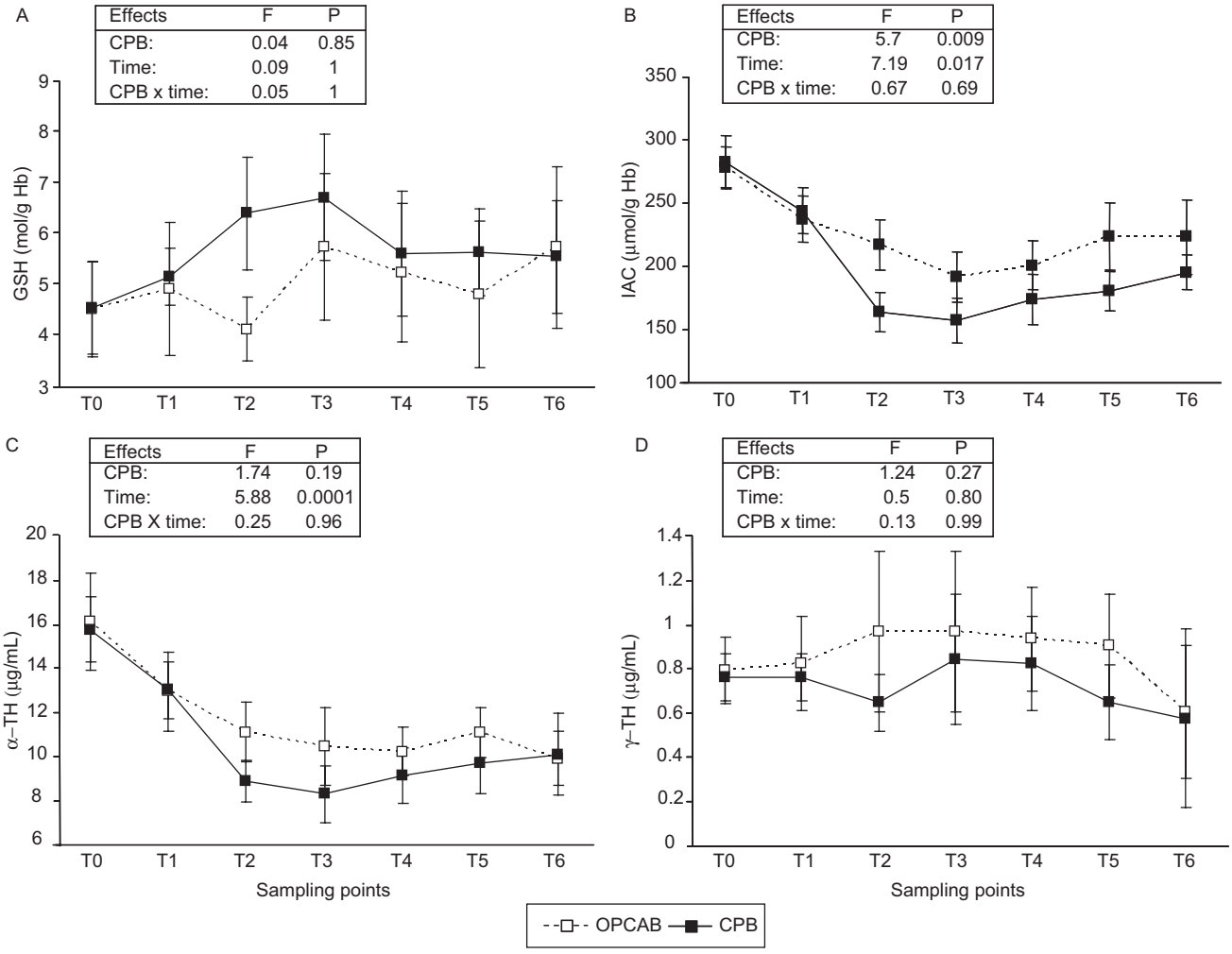

Figure 2. Time course of antioxidant biomarkers: reduced glutathione (GSH, panel A), individual antioxidant capacity (IAC, panel B), α-tocopherol (α-TH, panel C) and γ-tocopherol (γ-TH, panel D). Filled squares, solid line, cardiopulmonary bypass (CPB); empty squares, dotted line, off-pump procedure (OPCAB). Sampling points: T0, baseline; T1, after sternotomy; T2, 45 min after heparin administration; T3, after protamine administration; T4, at the end of surgery; T5, 4 h after arrival in the intensive care unit; and T6, 24 h after surgery. The values are expressed as means and 95% confidence intervals. Inserts show *F* and *p*-values for the effects of procedure (CPB), time and interaction of (CPB × time).

results of ANCOVA for the individual analytes and for OXYSCORE are reported in Figures 1–3. Changes of pro-oxidant biomarkers are shown in Figure 1. F-MDA (panel A), T-MDA (panel B) and $iPF_2\alpha$-III (panel C) significantly differed between CPB and OPCAB (treatment effect), varied with time (time effect) and the time courses for the two treatments were significantly different (treatment × time effect). No significant treatment, time or treatment × time effect were observed for GSSG (panel D).

Changes of antioxidant defence biomarkers (GSH, IAC, α-TH and γ-TH) are shown in Figure 2. Among these, significant treatment and time effects but no treatment × time effect were observed for IAC (panel B). A significant time effect was present for α-TH (panel C) and no effects were found for γ-TH (panel D) and GSH (panel A).

The OXY-SCORE increased markedly in CPB (Figure 3), peaking during the procedure (T3) with a sharp drop at the end of surgery (T4) followed by a progressive decline, like most of the pro-oxidant factors but, contrastingly, remaining well above baseline values even at 24 h. In OPCAB, a minor increase in OXY-SCORE was observed with values above baseline up to 24 h. Significant treatment, time and treatment × time effects were observed for OXY-SCORE.

Marker's ability to detect oxidative stress: variations vs baseline

The results for the paired-sample *t*-tests evaluating the within-subject variations in the two treatment groups are reported in Table 3. *p*-Values were corrected for multiple testing by the Bonferroni method. Whereas most of

the individual markers (with the exception of γ-TH, GSH and GSSG) showed significant changes during (T2–T4) CPB surgery, only T-MDA, α-TH and IAC were significantly altered during OPCAB, or after surgery (T6) in either CPB or OPCAB. The OXY-SCORE was significantly different from zero in either CPB or OPCAB, both during and after surgery. Notably, F-MDA was significantly altered in the OPCAB group at 24 h, but the levels were below baseline.

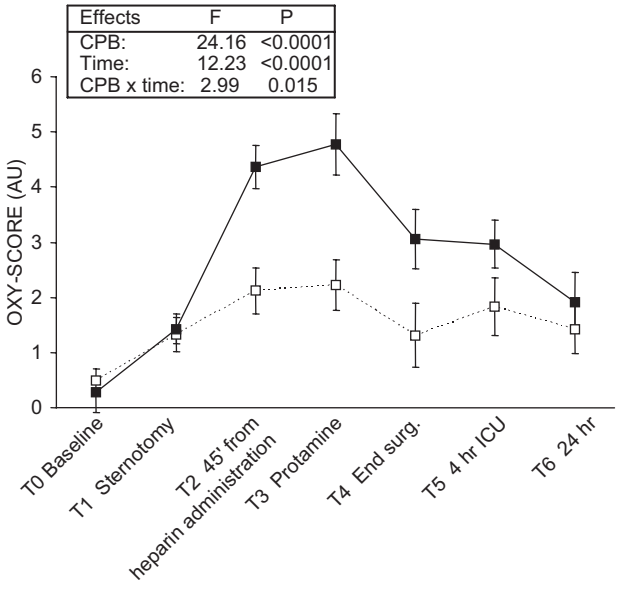

Figure 3. Time course of OXY-SCORE. Filled squares, solid line, cardiopulmonary bypass (CPB); empty squares, dotted line, off-pump procedure (OPCAB). The values are expressed as means and 95% confidence intervals. AU, arbitrary units. Inserts show *F* and *p*-values for the effects of procedure (CPB), time and interaction of (CPB × time).

Marker's ability to discriminate low-grade (OPCAB) from high-grade oxidative stress (CPB)

Table 4 shows the results of ROC curve analysis testing the ability of single markers and the OXY-SCORE to discriminate OPCAB from CPB. The discriminating ability was assessed at all time points. A value of the area under the ROC curve around 0.5 indicates complete lack of discriminating power (as in the case of all markers at T1); a value of 0.7 indicates a moderate and a value of 0.9 an excellent discriminating power. During surgery (T2–T4), the OXY-SCORE showed a high discriminating power, in line with the two forms of MDA; the discriminating power of isoprostanes and IAC was less apparent. In the postoperative period, the discriminating power of the OXY-SCORE was still moderately high, particularly at 24 h (T6).

Discussion

The present study shows that the global index OXY-SCORE, which summarizes data on oxidative damage

Table 3. Within-group comparisons of the different time points versus baseline.

	Sternotomy (T1)		45 min heparin admin. (T2)		Protamine (T3)		End surg. (T4)		4 h ICU (T5)		24 h (T6)	
	t-Value	*p*-Value	*t*-Value	*p*-Value	*t*-Value	*p*-Value	*t*-Value	*p*-Value	*t*-Value	*p*-Value	*t*-Value	*p*-Value
OPCAB												
Log $iPF_2\alpha$-III							-0.3	1.00			-1.6	0.73
F-MDA	-3.0	0.06	-0.9	0.986	-0.6	0.999	-1.5	0.77	-2.7	0.125	-3.5	**0.023**
T-MDA	0.6	1.00	3.1	**0.05**	3.3	**0.031**	3.9	**0.008**	7.7	**<0.001**	3.0	0.06
GSSG	1.3	0.88	0.5	1.00	-0.2	1.00	-1.4	0.83	0.9	0.99	1.4	0.81
GSH	0.7	1.00	0.4	1.00	1.9	0.47	1.2	0.92	0.5	1.00	1.8	0.57
α-TH	-4.8	**0.001**	-5.5	**<0.001**	-4.7	**0.001**	-5.3	**<0.001**	-5.0	**0.001**	-7.1	**<0.001**
γ-TH	0.5	1.00	1.0	0.97	1.3	0.90	1.4	0.82	1.0	0.97	-1.3	0.87
IAC	-5.2	**<0.001**	-7.7	**<0.001**	-11.7	**<0.001**	-9.8	**<0.001**	-4.6	**0.002**	-4.3	**0.003**
OXY-SCORE	4.2	**0.004**	5.8	**<0.001**	5.6	**<0.001**	3.0	0.06	5.0	**0.001**	3.8	**0.010**
CPB												
Log $iPF_2\alpha$-III							5.0	**0.001**			1.2	0.93
F-MDA	-0.2	1.00	4.6	**0.001**	9.6	**<0.001**	6.4	**<0.001**	2.0	0.436	-1.0	0.97
T-MDA	1.4	0.80	10.6	**<0.001**	12.3	**<0.001**	10.5	**<0.001**	7.2	**<0.001**	4.5	**0.002**
GSSG/HB	0.9	0.99	0.5	1.00	1.0	0.96	0.5	1.00	1.8	0.57	0.8	0.99
GSH/HB	2.7	0.12	4.0	**0.01**	5.8	**<0.001**	2.5	0.16	2.5	0.17	1.7	0.66
α-TH	-6.5	**<0.001**	-11.2	**<0.001**	-17.7	**<0.001**	-11.9	**<0.001**	-11.7	**<0.001**	-6.7	**<0.001**
γ-TH	0.2	1.00	-1.5	0.79	0.5	1.00	0.8	0.99	-1.3	0.90	-0.8	1.00
IAC	-6.4	**<0.001**	-15.0	**<0.001**	-18.5	**<0.001**	-12.3	**<0.001**	-10.6	**<0.001**	-9.6	**<0.001**
OXY-SCORE	6.2	**<0.001**	16.0	**<0.001**	19.6	**<0.001**	13.0	**<0.001**	11.3	**<0.001**	7.8	**<0.001**

p-Values are corrected for multiple comparisons by the Bonferroni method.
CPB, cardiopulmonary bypass; OPCAB, off-pump procedure; ICU, intensive care unit; MDA, malondialdehyde; IAC, individual antioxidant capacity; TH, tocopherols.

Table 4. Area under ROC curves.

	Sternotomy (T1)	45 min heparin admin. (T2)	Protamine (T3)	End surgery (T4)	4 h ICU (T5)	24 h (T6)	Median
Log $iPF_2\alpha$-III	0.50		**0.79**			0.67	0.67
F-MDA	0.63	**0.89**	**0.98**	**0.98**	**0.73**	0.64	**0.81**
T-MDA	0.56	**0.96**	**0.94**	**0.87**	0.52	0.59	**0.73**
GSSG	0.53	0.68	0.68	0.64	**0.74**	0.53	0.66
GSH	0.51	0.47	0.64	0.54	0.55	0.53	0.53
α-TH	0.49	0.68	0.68	0.62	0.63	0.40	0.63
γ-TH	0.48	0.63	0.59	0.56	0.64	0.44	0.57
IAC	0.50	**0.86**	**0.79**	**0.72**	**0.74**	0.67	**0.73**
OXY-SCORE	0.59	**0.89**	**0.92**	**0.88**	**0.77**	**0.70**	**0.82**

ICU, intensive care unit; MDA, malondialdehyde; IAC, individual antioxidant capacity; TH, tocopherols.

and on impairment of protective factors (Veglia et al. 2006), is able to depict accurately the time course of oxidative stress induced by coronary revascularization. Moreover, it exhibits a good discriminating ability in each time point, and the best discriminating ability overall, between two surgical approaches with different grades of oxidation.

We hypothesized that a comprehensive quantitative measure such as the OXY-SCORE might be more informative than the individual components of oxidative balance. The picture of the oxidative stress time course provided by the OXY-SCORE shows some worth mentioning dissimilarities when compared with those shown by individual damage biomarkers and antioxidant defences. Damage biomarkers (at least MDA and $iPF_2\alpha$-III) show a sharp increase during CPB and a nearly complete recovery afterwards. Levels of T-MDA remain significantly elevated, with respect to baseline, after 24 h. Conversely F-MDA levels are significantly below baseline at 24 h. In OPCAB, a minimal elevation during surgery is detected only by T-MDA. These three biomarkers exhibit a good discriminating power between the two procedures, but limited to the surgery period.

Among the markers of antioxidant defence, α-TH and IAC showed a significant change during surgery, but delayed in time with respect to damage markers. The alterations are still very significant at 24 h and are present in both groups. IAC, but not α-Th, exhibited a fair discriminating power, limited to the time span during and immediately after surgery. Alterations in the GSH–GSSG system showed a relatively low discriminating power, possibly due to the large variability in these markers, associated with the need to normalize on haemoglobin levels. The changes observed in individual markers are mostly in agreement with previous investigations in this field, showing an increased lipid peroxidation and a decreased antioxidant capacity during coronary bypass surgery, significantly more marked when cardiopulmonary bypass is performed (Gerritsen et al. 2001, Cavalca et al. 2006, Gonenc et al. 2006, Akila et al. 2007). Although the actual clinical significance of these differences is unknown, the extents of inflammatory as well as oxidative responses are putatively involved in determining a healthy recovery or the occurrence of complications after CABG (Biglioli et al. 2003). Moreover, whether administration of antioxidant compounds may favourably affect the oxidative balance and the clinical outcomes in these type of patients has yet to be experimentally explored. Therefore, the usefulness of different markers of oxidative stress is so far restricted to the research arena.

Yet, the marker that should be preferred to assess properly instant oxidative stress or oxidative changes has not been defined (Ridker et al. 2004, Dalle-Donne et al. 2006). The OXY-SCORE includes the characteristics of both damage and protection biomarkers. Specifically, in the description of the oxidative stress time course, the OXY-SCORE accounts for all of the following features: (1) a sharp rise during surgery in CPB; (2) a minor but significant increase in OPCAB, still maintaining an excellent power in distinguishing the two procedures; (3) the persistence of oxidative stress postsurgery, mainly in CPB but also in OPCAB; and (4) a detectable oxidative stress after 24 h, with a differential effect in the two procedures.

Different approaches to achieve a global index of oxidative stress in order to account for the manifold components of the oxidative balance were previously proposed. An integrated oxidative stress score has been proposed based on the estimated exposure to oxidant or antioxidant environmental factors (Wright et al. 2004, Goodman et al. 2007). Yet, in these scores, no evaluation of plasma or urine biomarkers was considered.

Another interesting approach combines plasma antioxidant and oxidant capabilities (Harma & Erel 2003, Kosecik et al. 2005, Kunt et al. 2006).

Specifically, Kunt et al. (2006) used an integrated oxidative stress index (OSI) including total peroxide concentration and total antioxidant capacity (analogous to IAC) to assess changes throughout surgical coronary revascularization. OSI increased steadily up to 40 h after surgery in both CPB and OPCAB. However, in contrast with the results obtained in this study using the OXY-SCORE and in spite of the several reports of differential

oxidative damage effects by CPB and OPCAB (Gerritsen et al. 2001, Cavalca et al. 2006, Gonenc et al. 2006, Akila et al. 2007), OSI did not discriminate between the two procedures during surgery and the two curves moderately diverged only at 24 h.

In summary, the OXY-SCORE is more informative than single markers and might discriminate better than other composite indexes between clinical conditions with different degrees of oxidative stress.

Acknowledgement

***Declaration of interest*:** Financial support was received from the Centro Cardiologico Monzino and the Italian Ministry of Health (Ricerca Corrente 2005, Progetto Bio 07). None of the authors had any personal or financial conflict of interest.

References

Akila, D'Souza B, Vishwanath P, D'Souza V. (2007). Oxidative injury and antioxidants in coronary artery bypass graft surgery: off-pump CABG significantly reduces oxidative stress. Clin Chim Acta 375:147–52.

Biglioli P, Cannata A, Alamanni F, Naliato M, Porqueddu M, Zanobini M, Tremoli E, Parolari A. (2003). Biological effects of off-pump vs. on-pump coronary artery surgery: focus on inflammation, hemostasis and oxidative stress. Eur J Cardiothorac Surg 24:260–9.

Cavalca V, Sisillo E, Veglia F, Tremoli E, Cighetti G, Salvi L, Sola A, Mussoni L, Biglioli P, Folco G, Sala A, Parolari A. (2006). Isoprostanes and oxidative stress in off-pump and on-pump coronary bypass surgery. Ann Thorac Surg 81:562–7.

Cherubini A, Ruggiero C, Polidori MC, Mecocci P. (2005). Potential markers of oxidative stress in stroke. Free Radic Biol Med 39:841–52.

Cighetti G, Debiasi S, Paroni R, Allevi P. (1999). Free and total malondialdehyde assessment in biological matrices by gas chromatography–mass spectrometry: what is needed for an accurate detection. Anal Biochem 266:222–9.

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. (2006). Biomarkers of oxidative damage in human disease. Clin Chem 52:601–23.

Droge W. (2002). Free radicals in the physiological control of cell function. Physiol Rev 82:47–95.

Gerritsen WB, van Boven WJ, Driessen AH, Haas FJ, Aarts LP. (2001). Off-pump versus on-pump coronary artery bypass grafting: oxidative stress and renal function. Eur J Cardiothorac Surg 20:923–9.

Gonenc A, Hacisevki A, Bakkaloglu B, Soyagir A, Torun M, Karagoz H, Simsek B. (2006). Oxidative stress is decreased in off-pump versus on-pump coronary artery surgery. J Biochem Mol Biol 39:377–82.

Goodman M, Bostick RM, Dash C, Flanders WD, Mandel JS. (2007). Hypothesis: oxidative stress score as a combined measure of pro-oxidant and antioxidant exposures. Ann Epidemiol 17:394–9.

Harma M, Erel O. (2003). Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly 133:563–6.

Kosecik M, Erel O, Sevinc E, Selek S. (2005). Increased oxidative stress in children exposed to passive smoking. Int J Cardiol 100:61–4.

Kunt AS, Selek S, Celik H, Demir D, Erel O, Andac MH. (2006). Decrease of total antioxidant capacity during coronary artery bypass surgery. Mt Sinai J Med 73:777–83.

Matata BM, Sosnowski AW, Galinanes M. (2000). Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. Ann Thorac Surg 69:785–91.

Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. (2004). Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation 109:IV6–19.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84.

Veglia F, Cighetti G, De Franceschi M, Zingaro L, Boccotti L, Tremoli E, Cavalca V. (2006). Age- and gender-related oxidative status determined in healthy subjects by means of OXY-SCORE, a potential new comprehensive index. Biomarkers 11:562–73.

Wang Z, Ciabattoni G, Créminon C, Lawson J, Fitzgerald GA, Patrono C, Maclouf J. (2007). Immunological characterization of urinary 8-epi-prostaglandin F2 alpha excretion in man. J Pharmacol Exp Ther 275:94–100.

Werba JP, Cavalca V, Veglia F, Massironi P, De Franceschi M, Zingaro L, Tremoli E. (2007). A new compound-specific pleiotropic effect of statins: modification of plasma gamma-tocopherol levels. Atherosclerosis 193:229–33.

Wright ME, Mayne ST, Stolzenberg-Solomon RZ, Li Z, Pietinen P, Taylor PR, Virtamo J, Albanes D. (2004). Development of a comprehensive dietary antioxidant index and application to lung cancer risk in a cohort of male smokers. Am J Epidemiol 160:68–76.